

iTenocyte-seeded Collagen Scaffold for Achilles Tendon Regeneration

Patricia Del Rio^{1,2}, Thomas Spaeter, PhD^{1,2}, Jacob Wechsler^{1,2}, Giselle Kaneda^{1,2}, Julia Sheyn^{1,2}, Dave Huang, MS^{4,5}, Oksana Shelest², Melissa Chavez^{1,2}, Victoria Yu^{1,2}, Melodie Metzger, PhD^{4,5}, Wafa Tawackoli, PhD^{1,2,3,5,6}, Dmitriy Sheyn, PhD^{1,2,3,5,6}

¹Orthopaedic Stem Cell Research Laboratory, ²Board of Governors Regenerative Medicine Institute, ³Department of Surgery, ⁴Orthopedics Biomechanics Lab, ⁵Department of Orthopedics, Cedars-Sinai Medical Center, ⁶Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA

Disclosures: P Del Rio (N), T Spaeter (N), J Wechsler (N), G Kaneda (N), J Sheyn (N), D Huang (N), O Shelest (N), M Chavez (N), V Yu (N), M Metzger (N), W Tawackoli (N), D Sheyn (N)

Introduction: Achilles tendon rupture accounts for 50% of musculoskeletal injuries. Low cellularity and low vascularization in the tendon cause the loss of biomechanical function and formation of scar tissue after an injury has been treated via surgery or physical therapy. Induced pluripotent stem cells (iPSCs) differentiated into induced mesenchymal stem cells (iMSCs) and overexpressing with Scleraxis (SCX), a tenogenic factor, have shown to induce tenogenic differentiation. We hypothesized that the iPSC-derived tenocytes (iTenocytes) seeded onto a biomechanically relevant collagen scaffold will regenerate the Achilles tendon biomechanical and functional properties after a full tendon injury.

Methods: This study was approved by Institutional Animal Care and Use Committee (IACUC). DuRepair collagen scaffold was biomechanically tested (Fig. 1a) and compared to native rat tendon tissue. iPSCs were differentiated into induced mesenchymal stem cells and transduced with SCX^{GFP+} lentiviral vector to create iTenocytes, labeled with DiI lipophilic dye and pre-seeded on collagen scaffold (Fig. 1b). Nude rats underwent a full 3mm Achilles tendon defect which was repaired with either suture only, scaffold only, or iTenocyte-seeded scaffold (Fig. 2a). The rats underwent gait testing at 2 weeks before surgery, at 96 hours, 3, 6, and 9 weeks after surgery. At 9 weeks, the rats were sacrificed, and tendon tissue was extracted for biomechanical testing and histological assessment.

Results: Wet DuRepair collagen scaffold demonstrated similar biomechanical properties when compared to native tendon tissue (Fig. 1a). Nude rats treated with iTenocyte-seeded scaffolds had a faster improvement in gait functionality when compared to groups treated with suture and scaffold only (Fig. 2b). Biomechanical outcomes of iTenocyte-seeded scaffold and scaffold only groups were comparable to native tendon tissue (Fig. 3a), but in terms of toughness, only seeded with iTenocytes DuRepair scaffolds and native tendons were significantly higher than suture only group. Histology demonstrated that the iTenocyte-seeded scaffolds resulted in a more organized tendon structure when compared to the cell-free scaffolds and suture only groups (Fig. 3b).

Discussion: This study demonstrate that iTenocyte-seeded collagen scaffolds can be potentially used as a treatment for regenerating the biomechanical properties and gait function of healing Achilles tendons.

Significance / Clinical Relevance: This study demonstrates that the combination of induced pluripotent stem cells and DuRepair collagen scaffold can regenerate the Achilles tendon after injury. This approach might revolutionize the use of stem cells and tissue engineering constructs as a potential treatment for tendon injuries.

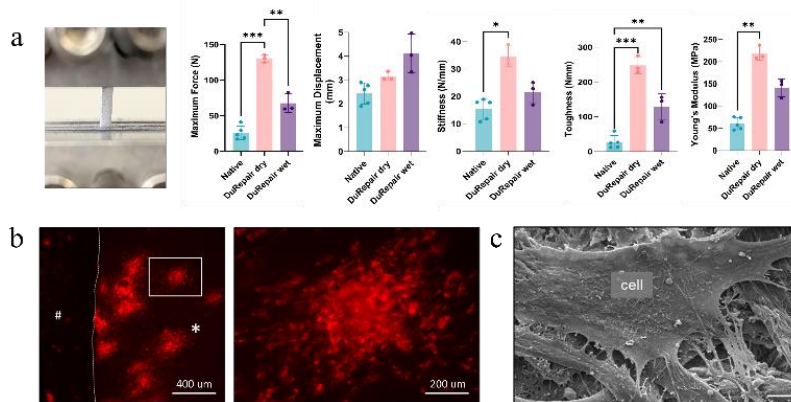


Figure 1: In vitro testing of collagen scaffold. Biomechanical testing of DuRepair collagen scaffold in dry and wet conditions compared to native tendon tissue (a). Fluorescent microscope images of DiI stained cells seeded onto scaffold (b). SEM images of scaffold seeded with cells (c). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (n=3)

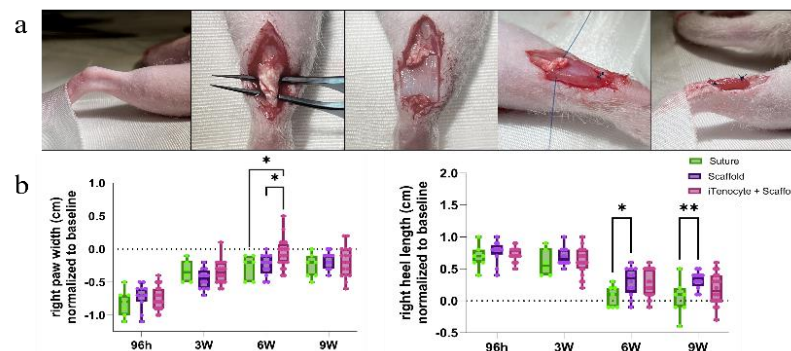


Figure 2: Achilles tendon injury and repair using collagen scaffold. Images demonstrating the position at which the right hind limb was placed to identify and isolate the Achilles tendon and create 3mm critical size defect and suture scaffold to the tendon (a). Gait analysis demonstrating improved gait functionality in iTenocyte-seeded scaffolds (b). *p<0.05, **p<0.01 (n=8-18)

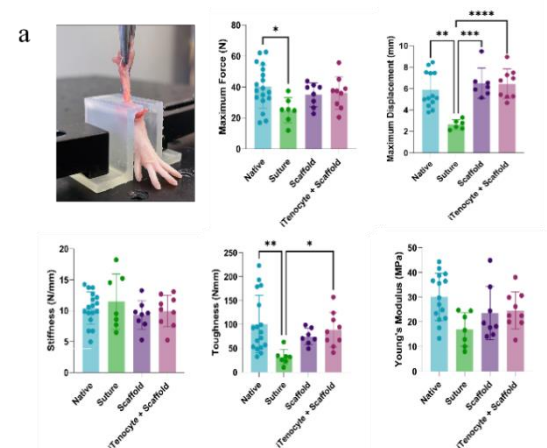


Figure 3: Biomechanical properties and structure of tendon has improved 9 weeks post injury and implantation of iTenocyte-seeded collagen scaffold comparing to control groups. Maximum load and displacement of iTenocyte-seeded scaffold and scaffold only groups were found similar to native tendon, but not to suture only (a). No significant differences were found in stiffness or Young's modulus, but the toughness was significantly improved in iTenocyte seeded scaffolds. iTenocyte-seeded scaffold demonstrated a more organized tissue when compared to other groups (b). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (n=8-18)